SCREENING OF XYLANASE PRODUCER FROM SOIL

WAN RAIMIE BINTI WAN RAMLI

A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2008

DECLARATION

"I declare that this thesis entitled 'Screening of Xylanase Producer from Soil' is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree",

Signature :…….
Name of candidate : Wan Raimie binti Wan Ramli
Date : 16 May 2008

DEDICATION

Special dedicated to my family, my friends, my fellow colleague,
and to all faculty members

For all your care, support, and believe in me.

Sincerely;
Wan Raimie binti Wan Ramli

ACKNOWLEDGEMENT

I would like to forward my very appreciation to my supervisor, Miss Wan Salwanis binti Wan Md Zain for the guidance and support. I also would like to express my gratitude to my academic advisor, Mr Mior Ahmad Khushairi bin Mohd Zahari for his support and believe in me during my studies.

I am very thankful to Universiti Malaysia Pahang and FKKSA for providing good facilities in campus and laboratory specifically. A very big thank you dedicated to all the staff of Faculty of Chemical and Natural Resources Engineering. Also not forgeting my family and colleagues.

ABSTRACT

The purpose of this study is to screen and to characterize the bacterial producing xylanase. The scopes include screening of xylanase producer and characterization of microorganisms and analysis of enzyme. This research was about an alternative to produce xylanase instead of using agricultural and forestry wastes, paper industry wastes, and various fruit wastes. The screening procedure was started from collecting soil sample until growth of bacteria on selective agar plate to screen the best xylanase producer from sample. After that, xylanase producer was characterized based on morphology using four types of morphological test. Besides that, the enzyme needs to be analyzed in different pH and temperature in term of protein content and xylanase activity. The optimum xylanase activity obtained was 72.667 U/ml at pH 6 and 65˚C.

ABSTRAK

Tujuan utama kajian ini adalah untuk memencilkan dan mencirikan bakteria yang menghasilkan enzim xylanase. Skop kajian termasuklah pemencilan bakteria yang menghasilkan xylanase dan pencirian bakteria dan enzim. Kajian ini adalah tentang alternatif lain untuk menghasilkan xylanase selain menggunakan sisa pertanian dan perhutanan, sisa daripada perindustrian kertas, dan sisa buah-buahan. Prosedur pemencilan bermula daripada pengumpulan sampel tanah sehingga pembiakan bakteria pada agar untuk pemencilan bakteria yang terbaik yang menghasilkan xylanase daripada sampel tanah tersebut. Selepas itu, bakteria yang menghasilkan xylanase dicirikan mengikut morfologi menggunakan empat jenis ujian morfologi. Selian itu, enzim xylanase juga telah dianalisa dalam pelbagai perbezaan pH dan suhu berdasarkan kandungan protein dan aktiviti xylanase. Keputusan kajian mendapati aktiviti xylanase optimum ialah 72.667 U/ml dalam pH 6 pada suhu 65˚C.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS/ABBREVIATIONS

ABS	-	absorbance
BSA	-	bovine serum albumin
cm	-	centimeter
g	-	gram
g/l	-	gram per liter
hr	-	hour
kD	-	kilodalton
mg	-	milligram
ml	-	milliliter
mm	-	millimeter
nm	-	nanometer
OD	-	optical density
rpm	-	rate per minute
sp.	-	species
sec	-	second
U/ml	-	Unit per milliliter
v/v	-	volume per volume
w/v	-	weight per volume
w/w	-	weight per weight
µg	-	microgram
µg g^{-1} hr^{-1}	-	microgram per gram per hour
µg/min/ml	-	microgram per minute per milliliter
˚C	-	degree Celsius
˚F	-	degree Fahrenheit
%	-	percent

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Introduction

1.1.1 Screening Xylanase Producer From Soil

Xylan is a polysaccharide found in plant cell walls. It is found in almost all parts of the plant. In plant biology, xylan is known as plant cell wall polysaccharide containing a backbone of β-1,4-linked xylose residues. Side chains of 4-O-methylglucuronic acid (glucuronoxylan) and arabinose (arabinoxylan) are present in varying amounts, together with acetyl groups. Xylan can be successfully applied to almost any clean, dry, oil-free surface. In fact, the only materials to which it will not adhere are those, which have inherent release characteristics such as polyolefins and fluoropolymers.

Endoxylanase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.8) are the main enzyme involved in xylan hydrolysis. Endoxylanase degrade plant cell wall polysaccharides by cleaving internal glucosidic bonds of xylan (Coughlan and Hazlewood, 1993). Xylanase deconstructs plant structural material by breaking down hemicellulose, a major component of the plant cell wall. Plant cell walls are necessary to prevent dehydration and maintain physical integrity. They also act as a physical barrier to prevent attack of plant pathogens. In nature, some plant consumers or pathogens use xylanase to digest or attack plants. Many microorganisms produce xylanase, but mammals do not. Some herbivorous insects and crustaceans also produce xylanase.

The xylanase enzyme (endo-1,4-β-xylanase, EC 3.2.1.8) from *Trichoderma* sp. has a pI of 9.0 and is produced by fermentation. Xylanase consists of 190 amino acids and has a molecular weight of 21 kD. Xylanases belong to the glucanase enzyme family and are characterized by their ability to break down various xylans to produce short-chain xylo-oligosaccharides. Xylanase readily crystallizes in ammonium sulfate and sodium/potassium phosphate across pH 3.5 to 9.0. Xylanase can also be crystallized with other salts, polymers, and organic solvents. Xylanase solubility increases with increasing temperature in moderate concentrations of ammonium sulfate. Xylanase solubility in phosphate pH 9 decreases in the temperature range of 0 to 10˚C but remains constant in the range of 10 through 37˚C. Xylanase has been extracted from many different fungi and bacteria. It is commonly used in animal feeds, paper production, and food production [2].

There are a few organisms that have been reported as xylanase producer such as *Bacillus circulans* (Heck *et al.,* 2005), *Enterobacter* sp. MTCC 5112 (Khandeparker and Bhosle, 2004), *Staphylococcus* sp. SG-13 (Gupta *et al.,* 2001), *Trichoderma harzianum* 1073 D3 (Seyis and Aksoz, 2005), and *Streptomyces* sp. (strain Ib 24D) (Rawashdeh *et al.,* 2005). *Bacillus circulans* and *Enterobacter* sp. MTCC 5112 are isolate from aquatic ambient while *Staphylococcus* sp. SG-13 is isolated from agricultural residues. *Trichoderma harzianum* 1073 D3 is isolate from natural wastes (orange pomace, orange peel, melon peel, hazelnut shell) but *Streptomyces* sp. (strain Ib 24D) is isolate from soil samples.

1.2 Problem Statement

In the last decade, production of xylanase enzyme has attracted the attention of many researchers, as these enzymes are essential for the degradation of plant biomass. Xylanases have potential applications in the pulp and paper, food, feed, and beverages industries. For commercial applications, xylanases should ideally be produce quickly and in large quantities from simple and inexpensive substrates. Natural xylan sources such as agricultural and forestry wastes, paper industry wastes, and various fruit wastes are potential raw material for xylanase production. As

xylanases have a wide range of application, economical production of these enzymes is of great importance (Seyis and Aksoz, 2005).

Fungi produced most of the enzymes. Due to the fact that fungi are hazardous, other methods in producing xylanase was found in bacteria. The bacteria can replace the fungi that give slower effect of degrading waste. Besides, bacteria are more compatible and robust compare to fungi. In this study, it is focus on bacteria in soil as a new source for producing xylanase. The bacteria will be cultivated to extract the enzymes needed and then being produced in a large quantity for industrial purpose in order to minimize raw material costs.

1.3 Objective

The objective of this study is to screen and to characterize the bacterial producing xylanase.

1.4 Scopes

The scopes of the research consist of three parts:

i) Screening of xylanase producer.

ii) Characterization of microorganism.

iii) Analysis of enzyme.

CHAPTER 2

LITERATURE REVIEW

2.1 Hemicellulose

Hemicellulose is a group of complex carbohydrates that, with other carbohydrates (*e.g.,* pectins), surround the cellulose fibres of plant cells. While cellulose is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous, structure with little strength. Hemicellulose consists of shorter chains compare to cellulose – around 200 sugars unit as opposed to 7000-15000 glucose molecules per polymer seen in cellulose. In addition, hemicellulose is a branched polymer. The most common hemicelluloses contain xylans (many molecules of the five-carbon sugar xylose linked together), a uronic acid (*i.e.,* sugar acid), and arabinose (another five-carbon sugar). Hemicelluloses have no chemical relationship to cellulose [8]. Hemicelluloses have the property of being soluble in dilute alkali. They are usually classified according to the sugar residues present xylan, mannans, arabinans, and galactans. Most hemicelluloses do not occur as homopolysaccharides but as in the side chain or appendages. These may be D-xylose, L-arabinose, D-mannose acid, O-acetyl groups or feruloyl and coumaryl ester linked via L-arabinose residues to the backbones (Coughlan, 1989).

2.1.1 Xylan

Xylan is a yellow, water-soluble, gummy polysaccharide found in plant cell walls and yielding xylose upon hydrolysis. Xylans can be hydrolyzed by β-xylanase. Xylan is the most abundant hemicelluloses, ranking second only to cellulose and constitutes up to 35% of the total dry weight plants. Xylans consist of a homopolymeric backbone of 1-linked α-D-xylopyranose units depending on its origin the backbone may be sustituted (Coughlan, 1989). The xylans are the major hemicelluloses of many plant materials where they often contribute to the rigidity of plant cell walls. Wood xylans are either O-accetyl-4-O-methylglucuronoxylans (in hardwoods) or arabino-4-O-methylglucuronoxylans (in softwoods). The degree of polymerization hardwoods xylan (150-200) is higher than that of softwoods (70-130) (Gray and Michael, 1991).

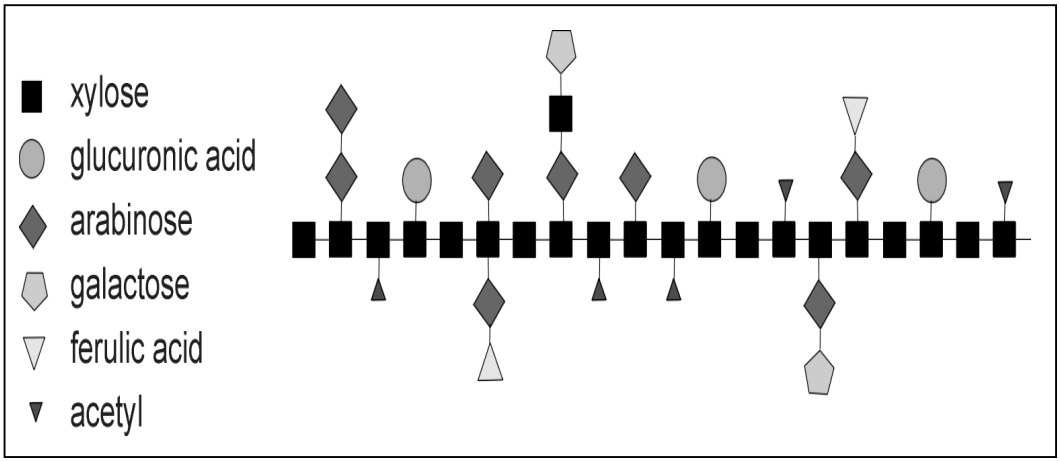

Figure 2.1 : Structure of the xylan [11]

2.1.2 Application of xylan

Xylan can be successfully applied to almost any clean, dry, oil-free surface. In fact, the only materials to which it will not adhere are those, which have inherent release characteristics such as polyolefins and fluoropolymers [12].

2.1.2.1 Metals

Almost every structural metal can be coated successfully with xylan, including steel (carbon and stainless), aluminum (wrought and cast), copper (and alloys), and titanium. High nickel- and chrome-bearing alloys, and some platings of nickel, can also be coated if abrasive blasting is used and the coatings are applied within an hour or two of preparation.

2.1.2.2 Plastics

Many plastic materials can be coated successfully with xylan, including nylon, PEEK, PEK, PPS, ABS, polycarbonate, epoxy, polyester and phenolic. The exceptions are the polyolefins and fluoropolymers - both of which have natural release characteristics. Also, vinyl products containing a high content of plasticizer can cause adhesion problems. Parts made of these materials must be cured at temperatures well below the softening temperature of the substrate to avoid distortion and polymer degradation.

2.1.2.3 Elastomers

Some xylan coatings may be applied successfully to elastomeric parts not expected to elongate more than about 30% in service. Elongation greater than this may cause the coating to crack. If a discontinuous coating is not objectionable, elongation far greater than 30% is permissible.

Elastomeric parts successfully coated with xylan include bushings, mounts, automotive door and window seals, and vibration dampers. Suitable substrates include natural rubber, EPDM, SBR, butadiene and its derivatives.

2.1.2.4 Glass and ceramics

Fluoropolymer coatings will adhere to clean ceramic or glass surfaces, but curing the coating without cracking the substrate can be difficult. (If possible, use glass or ceramic intended for high temperatures). In most cases, a low-temperature cure (below 150°C/300°F), followed by a slow cool-down period, will not crack the substrate. For glass parts, coating adhesion may be improved by fluorine.

2.1.2.5 Fabrics and composites

Xylan coatings are being increasingly used on woven and non-woven industrial textiles made from such modern materials as carbon fibre for low friction and release at elevated temperatures. One of the most successful applications of xylan involves a fabric bearing which is woven from a nylon/glass blend, then coated and cured.

These composite bearings are used under the compressor blades of large fanjet engines. The natural porosity of fabrics forms sponge-like "wells" into which the coating can penetrate. In service, this extra supply can continue to provide PTFE to a wear surface long after the coating has worn away from a smooth substrate. Xylan adheres well to other composites too, provided release agents have not been applied to the material.

2.1.2.6 Paper and wood

Xylan adheres well to uncoated or unvarnished paper products as well as wood. As unlikely as it may seem, the coatings perform every bit as well as they do on metal and other substrates. Cure temperature should not exceed 180°C/350°F.

2.2 Enzymes in Soils

Enzymes are proteins that act as catalysts by accelerating rates of reaction without undergoing permanent change. Enzymes are specific activators because they combine with their substrates in stereo specific fashion that decreases the stability of certain susceptible bonds, which reduces the energy of activation of reactions.

In soils, enzymes can exist intracellularly (inside the cytoplasmic membrane), which is of course important in cellular life processes. In addition, enzymes can exist outside the cytoplasmic membrane, in the periplasmic space or cell surface, and as extracellular enzymes in soil solution or stabilized in the soil matrix. It is generally assumed that soil enzymes are largely of microbial origin, but it is also possible that animals and plants may contribute enzymes to soils. The activity of approximately 100 enzymes has been identified in soils. The soil enzymes most often studied are oxidoreductases, transferases, and hydrolases. Some hydrolases and transferases have been extensively studied because of their role in decomposition of various organic compounds and thus are important in nutrient cycling and formation of soil organic matter. These include enzymes involved in: the C cycle, i.e., amylase, cellulase, xylanase, glucosidase, and invertase; the N cycle, i.e., protease, amidase, urease, and the aminase; the P cycle, i.e., phosphatase; and the S cycle, i.e., arylsulfatase.

A relatively small amount of any enzyme can be directly extracted from soil; therefore enzymes are mainly studied by measuring activity. The activity of enzymes varies temporally (seasonal), which often corresponds to microbial community responses to the environment, vertically (decreasing from the surface), at microscales, according to microbial community distribution, and at landscape level, where soil type is a major controlling factor (particularly textural distribution and organic matter).

Table 2.1 : The response of enzyme activities to the type of vegetation and soil (Daniel *et al.*, 2005)

Soil enzyme activity	Range of activities	Vegetation/soil type
Xylanase activity	13-24 (mg glucose g^{-1} per 24hr)	Spruce forest/n.k.
	0.28-8.0 (mg glucose g^{-1} per 24hr)	Beech forest/n.k.
	1.8-3.0 (mg glucose g^{-1} per 24hr)	Grassland/orthic luvisol
	0.24-1.83 (mg glucose g^{-1} per 24hr)	Agricultural land/haplic luvisol,entisol
β-Glucosidase	20-55 (μg ρ-nitrophenol g^{-1} hr^{-1})	Grassland/pachic arguistoll
	36-160 (μg ρ-nitrophenol g^{-1} hr^{-1})	Forest/Haplohumult
	130-310 (μg ρ-nitrophenol g^{-1} hr^{-1})	Crop rotation/hapludalf
	71-86 (μg ρ-nitrophenol g^{-1} hr^{-1})	Crop rotation/pachic ultic argixerolls
	41-253 (μg ρ-nitrophenol g^{-1} hr^{-1})	Crop, manured soil, pasture/typic haploxeroll
Protease activity	150-520 (μg tyrosine g^{-1} per 2 hr)	Agricultural land/haplic chernozem
	224-514 (μg tyrosine g^{-1} per 2 hr)	Pasture/typic dystrochrept
	120-430 (μg tyrosine g^{-1} per 2 hr)	Wheat seeds/loamy sand
	198-288 (μg tyrosine g^{-1} per 2 hr)	Crop rotation/haplic luvisol
Arginine deaminase activity	2.5-5.0 (μg N g^{-1} hr^{-1})	Grassland/pachic arguistoll
	1.7-2.0 (μg N g^{-1} hr^{-1})	Crop rotation/phaeozem, lithosol, cambisol
	4.0-11.0 (μg N g^{-1} hr^{-1})	Forest/sandy soils
	0.1-1.3 (μg N g^{-1} hr^{-1})	Crop rotation/fluventic ustochrept
Arylsulfatase activity	30-50 (μg ρ-nitrophenol g^{-1} hr^{-1})	Grassland/pachic arguistoll
	115-340 (μg ρ-nitrophenol g^{-1} hr^{-1})	Agricultural land/hapludoll
	6.9-213 (μg ρ-nitrophenol g^{-1} hr^{-1})	Pasture/typic dystrochrept
	21-49 (μg ρ-nitrophenol g^{-1} hr^{-1})	Forest/podzol
	12-58 (μg ρ-nitrophenol g^{-1} hr^{-1})	Crop, manured soil, pasture/typic haploxeroll
Alkaline phosphatase	40-80 (μg ρ-nitrophenol g^{-1} hr^{-1})	Grassland/pachic arguistoll

	40-790 (μg ρ-nitrophenol g^{-1} hr^{-1})	Agricultural land/aeric vertic epiaqualfs
	100-500 (μg ρ-nitrophenol g^{-1} hr^{-1})	Crop rotation/hapludalf
	181-225 (μg ρ-nitrophenol g^{-1} hr^{-1})	Crop rotation/ustochrept
Dehydrogenase	114-155 (μg TPF g^{-1} 24 hr)	Crop rotation/haplumbrepts, hapludalfs
	0.6-0.9 (μg TPF g^{-1} 24 hr)	Crop rotation/fluvisol
	68-97 (μg TPF g^{-1} 24 hr)	Crop rotation/ustochrept
	148-207 (μg TPF g^{-1} 24 hr)	Crop rotation/fluventic xerochrept

n.k., soil type not known

2.2.1 Xylanase

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide β-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants. As such, it plays a major role in the digestive system of herbivorous microorganisms (mammals, conversely, do not produce xylanase). Additionally, xylanases are present in fungi for the degradation of plant matter into usable nutrients. Commercial applications for xylanase include the chlorine-free bleaching of wood pulp in the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting). Additionally, it is the key ingredient in the dough conditioners s500 and us500 manufactured by Puratos. These enzymes are used to improve the dough's workability and absorption of water. In the future, xylanase may be used for the production of biofuel from unusable plant material (William, 1997).

Xylanase is used to break down plants as well as the sugar xylose. It has been found in many different fungi and bacteria. The enzyme is from *Trichoderma* sp. and consists of 190 amino acids. Xylanase belongs to the glucanase enzyme family, which is characterized by their ability to break down various xylans to produce short-chain xylo-oligosaccharides. Enzymes are important for a proper